**MANGALORE UNIVERSITY**

**PhD FINAL SYNOPSIS**

# "STUDIES ON HUMAN SECRETED PHOSPHOLIPASE $A_2$ IN CANCER MANAGEMENT"

***In fulfilment of the requirement for the degree of***
*Doctor of Philosophy in Biochemistry*


**Submitted by**
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Registration no: MU/ACC /Ph.D-Reg/CR-219/2015-16/A3

Under the Guidance of
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## Introduction

Inflammation is a defensive process to all sorts of tissue injury that involves the action of several enzymes and chemical mediators at local and systemic levels. Lipid derivatives from membrane glycerophospholipids are produced at a fairly high rate during inflammation. Along with these lipid mediators, various free radicals such as superoxide and hydroxyl radicals are produced. Phospholipase $A_2$ are a class of enzymes that play a regulatory role in inflammation which catalyse the hydrolysis of membrane glycerophospholipids to release arachidonic acid and lysophospholipids. Arachidonic acid is the precursor for generation of pro-inflammatory mediators such as prostaglandins, leukotrienes, thromboxanes and platelet activation factors. Inhibition of this reaction is of intensed pharmacological interest because the release of arachidonate is the rate-limiting step in the production of eicosonoid mediators and various free radicals. The ROS released during the eicosanoid pathway in turn activate $PLA_2$ enzyme and thereby worsen inflammatory condition. Therefore molecules with antioxidant activity and having $PLA_2$ inhibitory activity are useful in treating various inflammatory diseases.

Literature surveys reveal that secondary metabolites such as alkaloids, flavonoids, triterpenes and napthoquinones from plants are potent anti-inflammatory agents. Many flavonoids and terpenoids having antioxidant activity are found to act as $PLA_2$ inhibitors. Attempts have been made against inflammation based on the ethnopharmacological importance; targeting $PLA_2$, the key enzyme of inflammation. Medicinal plants such as *Annona glabra, Celtis timorensis, Centella asiatica, Ficus tsjakela, Melastoma malabathricum, Phyllanthus niruri, Spondias pinnata and Ziziphus maurtiana* were screened for $PLA_2$ inhibition. Among them, *Centella asiatica and Melastoma malabathricum* have shown greater sPLA2 inhibitory activity and antioxidant activity. Corosolic acid and quercitrin are the anti-inflammatory molecules from *Centella asiatica and Melastoma malabathricum* were procured and tested for antioxidant activity, $PLA_2$ inhibition and anticancer activities.

## Aim and scope of the present investigation

There are many ways to bring down inflammation and its damage to the human system. Glucocorticosteroids and non-steroidal anti-inflammatory agents have been used for decades as anti-inflammatory drugs even though they have considerable side effects on the human body. Scientific teams around the globe are trying to discover specific $PLA_2$ inhibitors which act as therapeutically important anti-inflammatory molecules. $PLA_2$ inhibitors prevent the release of arachidonic acid at regulatory step and bring down the concentration of proinflammatory mediators like prostaglandins, leukotrienes, lipoxins and platelet-activating factors. Clinical results demonstrate that inhibitors of $PLA_2$ enzyme are more effective in developing a therapeutically potent anti-inflammatory drug as powerful as glucocorticosteroids. Therefore, inhibitors targeting $sPLA_2$ IIA from the folk medicinal plant are in demand.

There are many plants with anti-inflammatory properties used by local medicinal practitioners around the world that appear to have no scientific validation for their use in most cases even though they are found to be effective. This thesis reports the activity-guided screening of $sPLA_2$ inhibitors from plants on the basis of their previous antiinflammatory reports. Identified $PLA_2$ inhibitors are subjected to biophysical and biochemical investigation for observed inhibition. In addition, the *in situ* indirect hemolytic activity and *in vivo* edema-inducing activity of the $sPLA_2$ enzyme were neutralized to establish the potency and usefulness of the compounds as anti-inflammatory and anticancer activity.

## Objectives

- Screening and extraction of medicinal plant/s for $PLA_2$ inhibition.
- Identification/isolation of $PLA_2$ inhibitor/s from plant extracts to evaluate in vitro anti-inflammatory and anti-cancer activities.
- Evaluation of *In vivo* $sPLA_2$ inhibition for anti-inflammatory and anticancer activities.
- Elucidation of molecular mechanism of $PLA_2$ inhibition underlying anti-inflammatory and anticancer activities of novel compounds.
- Bioinformatics studies of $sPLA_2$ inhibition by identified/isolated molecules.

The thesis is presented in four chapters followed by the summary and conclusion, bibliography.

**Chapter-I**

Chapter I is an introductory chapter that gives a review of literature on the inflammation that deals with complex ways and means by which it is brought about in animal systems. The role of $PLA_2$ enzymes, their classification, and why they are targeted for inhibition are explained in detail with the help of references and about NSAIDS. This chapter also provides detailed information about different $sPLA_2$ inhibitors, inflammation linked with prostate cancer, the role of IL-6 in inflammation and cancer.

**Chapter-II**

Chapter II describes the materials and methods for purification of venom $PLA_2$s, antioxidant activity (DPPH, phosphomolybdenum and anti-lipid peroxidation), $sPLA_2$ inhibition, inhibition of indirect hemolytic activity and edema inducing activity of $sPLA_2$, biophysical experiments such as fluorometric and CD interaction, anticancer activities (MTT assays, caspase activity and anti-tumor activity), IL-6 expression in EAC induced mice.

**Chapter-III**

Chapter-III is all about the inhibition of $sPLA_2$ enzymes by different plant extracts. Out of eight plants chosen for the study, *Centella asiatica* and *Melastoma malabathricum* showed higher antioxidant activity, sPLA2 inhibition (*in vitro* and *in vivo*) and anticancer activity. The plants showed desired properties chosen for the identification/isolation of the bioactive active principles for observed activities of crude extracts.

**Chapter-IV**

The most active compounds like corosolic acid and quercitrin were found predominantly in plants *Centella asiatica* and *Melastoma malabathricum* were procured and subjected for the inhibition of $sPLA_2$ IIA, determination of antioxidant activity and anti-cancer activity. The results of $sPLA_2$ inhibition, antioxidant and anticancer activity of inhibitors are correlated to

the results of crude extracts. The biochemical and biophysical interactions of corosolic acid and quercitrin with $sPLA_2$ enzymes was also studied.

## Summary and Conclusions

The literature survey revealed that $PLA_2$ is a growing family of phospholipid hydrolyzing enzymes existing in different isoforms. Based on their structural aspects and catalytic mechanism these enzymes are classified. Among these groups, IIA $sPLA_2$ is mainly responsible for the synthesis of various inflammatory mediators hence is chosen for our study. Some of the important medicinal plants were selected based on traditional use to screen $PLA_2$ inhibitors. Many of the protective biological effects of plants are due to their antioxidant contents and that is the scientific grounds for using plant food as medicine.

Among medicinal plants screened for $sPLA_2$ inhibition, *Centella asiatica* and *Melastoma malabathricum* showed the highest inhibition of $sPLA_2$ activity and exhibited good antioxidant and anti-cancer activity. The major compounds such as corosolic acid and quercitrin rich in the above medicinal plants were procured and checked for different pharmacological properties. The compounds corosolic acid and quercitrin inhibited sPLA2 in a dose-dependent manner and $IC_{50}$ value 7.405378 µg/mL and 4.861751µg/mL respectively. Corosolic acid and quercitrin have shown antioxidant activity to a good extent. Inhibition of $sPLA_2$ activity by corosolic acid and quercitrin was independent of calcium (100 to 500 µM) and substrate (5 to 30 nmol) concentration. The biophysical studies, corosolic acid and quercitrin quenched the relative fluorescence intensity of $sPLA_2$ in a dose-dependent manner. In the presence of these inhibitors, an apparent shift in the far UV-CD spectra of $sPLA_2$ was observed that implied the direct interaction with the enzyme. Inhibition of $sPLA_2$ induced mouse paw edema and indirect hemolytic activity by corosolic acid and quercitrin confirms its $sPLA_2$ inhibitory activity *in vivo* and *in situ* respectively. The inhibitors also exhibited moderate anti-cancer activity on the PC-3 cell line.

The concomitant inhibition of *in-vitro* $sPLA_2$ activity and *in vivo* edema inducing activity by corosolic acid and quercitrin indicates the correlation between lipolytic and pro-inflammatory activities. Corosolic acid and quercitrin are excellent anti-inflammatory molecules exhibiting pharmacological action by inhibiting the key enzyme $sPLA_2$. In addition,

due to antioxidant activity of corosolic acid and quercitrin may thus scavenge the free radicals released and free radical intermediates during arachidonate metabolism. Therefore, these molecules form a new class of $sPLA_2$ inhibitors with antioxidant and anticancer activity from medicinal plants. Further clinical experiments may help to design corosolic acid and quercitrin as useful anti-inflammatory drugs.

## Paper Publication

- **P. Sophiya**[1], B. KiranKumar[2], N.S. Lohith[1], Fasil Ali[1], A.D. Sathisha[3] and K.K. Dharmappa[1]. GC-MS analysis, ADME toxicity and *in-silico* studies of some isolated compounds from *Melastoma malabathricum* leaves against $sPLA_2$ Inhibition. Applied biological research. Accepted for publication in **2021**. DOI:10.5958/0974-4517.2020.00000.00
- **Sophiya P**, Lohith N. S. Dharmappa KK*****. Study of phytochemical analysis and evaluation of antioxidant, anti-inflammatory function by GIIA $sPLA_2$ inhibition by leaves of *Annona glabra*: An approach to treat the inflammatory phase of cancer. *Biomedicine*;(40)01**,2020.**
- **P. Sophiya,** N. S. Lohith, A. S. Giresha, M. Narayanappa, Rajkumar S. Meti, A. D. Sathisha, and Dharmappa KK*****. Inhibitory effect of ethanol extract of *Melastoma malabathricum* leaves on inflammatory $sPLA_2$ enzyme and its ability to reduce the cell viability of PC3 cell line. *International Journal of Pharmaceutical Sciences and Research*; 10(11): 5041-50. **2019.**
- N Shobha, N Nanda, Aladahalli Shivanna Giresha, Praveen Manjappa, **P Sophiya**, Dharmappa K. Kand B.M. Nagabhushana.Synthesis and characterization of Zinc oxide nanoparticles utilizing seed extract of *Ricinus communis* and study of its antioxidant, antifungal and anti-cancer activity. *Materials Science and Engineering: C*; 97, 842-850, **2019.**
- Sathisha A. D., Hemakumar K.H.**, Sophiya P.** Giresha A.S., Lohith N S, Basavaraju Y. B .and Dharmappa K.K. Synthesis, anticancer and antimitotic activity of analogues of podophyllotoxin on b16f10 melanoma cell lines and Allium cepa L.Biomedicine.

- **Book Chapter :** Chapter : **Sophiya P**[1], Narayanappa M[1],Deepadarshan Urs[1], Devaraju KS[2], Shankar J[3]and Dr. K K Dharmappa[1] *Medicinal and nutritional aspects of wild edible fruits from Kodagu. Bentham publisher (Under review).
- **Book Chapter:** Chapter: **Sophiya P**, Deepadarshan Urs, Nagma Banu H. A. Rajkumar S. Meti, Shankar J and Dr. K K Dharmappa[1] * Advanced voltammetric devices for disease detection Bentham Science publishers pte ltd (Under review).

## Conference/Symposium/Seminar/Workshop

- **International Conference** on Advances in pharmaceutical and Health sciences (ICAPHS-2020) held from 20th-22nd February 2020 at K. S. Hegde Auditorium, Nitte (Deemed to be university), Deralakatte, Mangalore.

  **Oral presentation** Title: Evaluation of secretory phospholipase A2 inhibition by medicinal plant *Celtis timorensis*
- **National conference** on Recent Novel approaches in chemical Sciences held on 12th February 2020, organized by the department of chemistry, Field Marshal K. M. Cariappa College, Madikeri.

  **Oral presentation** Title: Inhibition of secretory phospholipase $A_2$ by *Ficus tsjakela* BURM. F: An approach to neutralize inflammatory phase of cancer.
- **International symposium** on 'Discovery of actionable targets in infectious disease' organized by Center for System Biology and molecular medicine, Yenapoya Research Centre, Mangalore, held on 26 November **2019**.

  **Poster Presentation:** 'Puerarin a potent anti-inflammatory agent through modulating $sPLA_2$ IIA isolated from human pleural fluids of tuberculosis patients'.
- Participated in the international workshop on biology and applications of actinomycetes organized by University of Mysore, Mysore, India in association with Helmholtz Centre for infection Research & Technical University of Braunschweig, Germany held at university of Mysore, Mysore, India, during 31st October -1st November 2019.

- **International Conference** on Impact of Innovations in science and technology for societal development (IISTSD-2019) held during 19-21st September 2019 at kongunadu arts and science college (Autonomous), Coimbatore, Tamil Nadu, India.

  **Oral presentation: Best oral presentation Award: Evaluation of anti-inflammatory function of *Annona glabra* by GIIA sPLA$_2$ inhibition: An approach to treat inflammatory phase of cancer'**
- Participated in Citizen science research education programme (CSREP) of ANCF in collaboration with Institute of wildlife Veterinary Research, KVAFSU, WGNF, CWS, KMFT, WRRC and friends of elephants one day regional seminar on Biodiversity titled " Health and wealth of wildlife in Coorg, held on 25th June 2019 at the institute of wildlife veterinary research, Kodagu, Karnataka.
- **International conference** on Advanced Functional Materials for Energy, Environment and Health Care (AFMEEHC) organized by University with Potential for Excellence (UPE), University Mysore, held on 18-20 March **2019.**

  **Paper presented**: Green synthesis and characterization of gold and silver Nanoparticles of *Caesalpinia bonducella* leaves extract and evaluation of their antibacterial, antifungal, phytochemical analysis and larvicidal activity.
- **National conference** on Recent Advances in material science held on 5th February 2019 organised by the department of chemistry, field marshal K. M. cariappa college, Madikeri.

  **Paper presented**: Neutralization of neuro-inflammation by sPLA$_2$ IIA inhibition from ethanol extract of *Myristica fragrans.*
- Attended Dr. Sarada subramaniam memorial Pre-conference **Workshop** on "Basics of Cell Culture and Applications in Drug Discovery". On 15th November 2018. at science block post graduate centre , Jnana Kaveri, Chikka Aluvara, Kodagu , Karnataka India.
- **39th Annual conference** of Indian association of biomedical scientists (IABMS) and International Conference on 'Current concepts on the role of Indian medicine and phytoceuticals in the maintenance of health' organised by Jnana Cauveri Post Graduate campus Mangalore University, Chikka Aluvara, Kodagu, held on 15 - 17 November **2018.**

**Papers presented:** Leaves of *Melastoma malabathricum* for $sPLA_2$ inhibition in inflammation and cancer.

- **National conference** on 'Natural bioactive compounds and health care' organized by department of Biochemistry, Mangalore University, Jnana Cauveri campus, Kodagu sponsored by DST in association with UGC and SBC held on 6 to 7 October **2017.**

  **Poster presentation:** Evaluation of efficacy of $sPLA_2$ inhibitors in down regulation of ERK1/2-cPLA2 pathway in esophageal adenoma cell growth and cancer.
- Presented a Poster in the UGC sponsored **state level conference** on frontiers in life science 2017 at government science college Hassan on 28th March 2017.

  **Poster presentation:** 'Inhibition of secretory phospholipase $A_2$ from human synovial fluid by ethanol extract of *Averrohoea bilimbi* as an anti-inflammatory activity'
- One day National level conference on frontiers in Biotechnology & Life Science on March 3rd 2017 at SDM Educational Society Ujire, MMK and SDM Mahila Mahavidyalaya, Mysore.

  **Poster presentation: Best poster presentation award 'Human pleural fluid phospholipase $A_2$ inhibition by methanol extract of *Mimusops elangi*: Neutralization of inflammation'.**

## Webinar Series

- Participated in the [PacBio APAC webinar Series] Advancing Pharmacogenomics research and the need for highly accurate Long Read Sequencing , June 24th 2021.
- Participated in the national webinar on "Human fungal infections with special reference to mucormycosis" held on June 10th, 2021organized by Department of Microbiology B. M. S. College for women, Bangalore, Karnataka.
- Participated in the Faculty development program on "fundamentals of In Vitro Animal cell culture techniques organised by the department of Biotechnology, under the aegis of The oxford Science Skill enhancement centre (TOSSEC)", the Oxford Science College of Science, Bengaluru, Karnataka, India, held from 3rd to 7th May 2021.

- Participated in the national level webinar series on COVID-19 held from May 03 to May 07, 2021 organized by Mysore science foundation Affiliated to vigyan prasar club id: VPKA0018, Mysore Karnataka.
- Participated in the International webinar on " The Career Catalyst: Plan, Prepare & Prosper in Life Sciences" organized by the division of Biochemistry, Faculty of Life Sciences, JSS AHER, Mysuru, held on 8th and 9th May 2021.
- Participated in a one-day International webinar on "Future Prospective of Biotechnology" held on 27th April 2021, organized by the department of post-graduation studies and research in biotechnology, Gulbarga University, Kalaburgi. Certificate ID : VoLMG-CE000050
- Participated in the SPSR national webinar on "Role of pharmacists for best therapeutic outcomes" organized by Society of pharmaceutical sciences and research (SPSR) on 29th November 2020.
- Participated in the two days International e conference on new horizons in biochemistry, Microbiology and food technology -2020 jointly organized by Yogi Vemana University & University Malaysia Kelanatan held on October 12th and 13th 2020.
- Participated in the International e conference series on "Advances in chemical sciences, health and education on the topic 'Instruments used in advanced chemical analysis and material characterization organized by the department of chemistry, I.D.S.G Government college, Chikkamagaluru -577102, India on 09.11.2020.
- Participated in the International e conference series on "Advances in chemical sciences, health and education - on the topic Cyber space and its safe organized by the department of chemistry, I.D.S.G Government college, Chikkamagaluru -577102, India on 10.11.2020.
- Participated in the International e conference series on "Advances in chemical sciences, health and education on the topic 'Impact of chromatography in the pharmaceutical industry organized by the department of chemistry, I.D.S.G Government College, Chikkamagaluru -India on 05.11.2020.
- Participated in the 'International webinar on "COVID 19: current status of vaccine development and Immunization' jointly organized by Mangalore University and microbiologists society, India (MSI), Karnataka chapter, held on 30th September 2020.

- Advances in chemical sciences, health and education on the topic Impact of chromatography in the pharmaceutical industry organized by the department of chemistry, I.D.S.G Government College, Chikkamagaluru, India on 05.11.2020.
- Participated in the webinar on computer assisted new chemical structure elucidation-From ACD organized by Dept. of studies in organic chemistry, Manasagangothri, Mysore on 21st September 2020.
- Participated in the International webinar on 'Recent trends and challenges in neuroimmunology organized by P G Dept. of studies in Biochemistry, Karnataka University Dharwad, from 10th August to 13th August 2020.
- Participated in international webinar series on De-covidizing Biotechnology research 2020 organised by the Department of Biotechnology and Bioinformatics at Kuvempu University Jnanasahyadri, Shankaraghatta, Shivamoggha Dist, Karnataka from 10.08.2020 to 14.08.2020.
- Participated in one day 'International webinar on Recent trends in plant tissue culture and eco-biodiversity conducted by the department of Botany and En-Sciences, research laboratory of applied biological Science, under the aegis of IQAC, DVS college of arts, Science and commerce , Shivamogga on 14th August 2020
- Participated in an international virtual conference on opportunities and challenges in preclinical drug Discovery organized by NGSM institute of pharmaceutical sciences held on 4th July 2020

**Ms. Sophiya P** **Dr. K.K Dharmappa**

Research Student Research Supervisor

Place: Chikka Aluvara
Date: 25 January 2021

## Ethical Clearance Certificates

**MANGALORE UNIVERSITY**

**INSTITUTIONAL HUMAN ETHICS COMMITTEE**


**Chairman:**
Dr. M. S. Ravi
Professor and Head,
Dept. of Orthodontics
A.B. Shetty Memorial Institute of Dental Sciences, Nitte University

**Members:**

Dr. Amrith M. Mirajkar
Professor and Head,
Dept. of Physiology
K. S. Hegde Medical Academy, Nitte University

Dr. K. S. Sreepada
Professor,
Dept. of Applied Zoology,
Mangalore University

*Dr. Patric D'Souza*

Dr. Nazeer Rahim Attar
Professor,
Dept. of Medicine, Yeneopoya University

Mr. Udayananda
Advocate

Dr. Mohan S. Singhe
Asst. Prof.,
Dept. of Social Work, Mangalore University

Mr. Ahmed Bawa

**Member Secretary:**
Dr. Monika Sadananda
Professor,
Dept. of Biosciences, Mangalore University

ihec.mu@gmail.com


No.**MU-IHEC-2018-1** Date: 25.10.2018

To
Dr. K. K, Dharmappa
Department of Biochemisty
Mangalore University PG Centre

CERTIFICATE

This is to certify that the proposal of your PhD student Ms. Sophiya P. entitled "**Studies on human secreted phospholipase A2 in cancer management**" submitted to the Institutional Human Ethics Committee was presented and discussed in the meeting. Subsequent to incorporation of the suggestions given by the IHEC, the Committee has approved the revised proposal.

You are permitted to carry out the research work with your student **Ms. Sophiya P.** in compliance with the revised proposal submitted to the IHEC, Mangalore University and in accordance with the guidelines thereof laid down by ICMR.

You are required to submit the progress report of the project periodically to the Committee for review.

**Dr. M. S. Ravi**
Chairman

NITTE (Deemed to be University)
NGSM INSTITUTE OF PHARMACEUTICAL SCIENCES
(Established under Section 3 of UGC Act, 1956)
Placed under Category 'A' by MHRD, GoI, Accredited with 'A' Grade by NAAC

Ref: NGSM/IAEC/2019-20/167 Date: 18th November 2019

To

Ms. Sophiya P.
Research Scholar
Jnana Kaveri Post Graduate Centre, Mangalore University
Chikka Aluvara, Kodagu, Karnataka-571232

**Sub: Approval of protocol by IAEC**

The Institutional Animal Ethics Committee of NGSM Institute of Pharmaceutical Science has approved your protocol for experimentation on small animals for the project titled **"Studies on Human Secreted Phospholipase $A_2$ in Cancer management"** during the meeting held on 9th November, 2019. Approval number of your protocol is **NGSMIPS/IAEC/NOV-2019/166.**

**Chairman and Scientist In-charge of Animal House Facility**
Dr. C. S. Shastry
Principal

PRINCIPAL
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**Certificates**

MMK & SDM Mahila Maha Vidyalaya

Krishnamurthypuram, Mysore

ONE DAY NATIONAL LEVEL CONFERENCE ON

"FRONTIERS IN BIOTECHNOLOGY & LIFE SCIENCES"

Sponsered by: Sri. Dharmasthala Manjunatheshwara Educational Society, Ujire

CERTIFICATE

This is to certify that ~~Mr./Ms./Dr/Prof~~ SOPHIVA . P

of REGEARCH GCHOLAR BIOCHEMISTRY College P.G CENTER, KUSHALNAGAR

has ~~participated as a Resource person / Delegate~~ SECURED I PRIZE IN POSTER PRESENTATION in the National Level Conference held on 3rd March 2017

organized by the Department of Biotechnology of our College.

PRINCIPAL

Smt. Anupama.R
Convener

Prof.K.V.Damodara Gowda
Principal

SERB, Govt. of India and Indian Science Congress Association, Coimbatore Chapter - Sponsored

International Conference on

IMPACT OF INNOVATIONS IN SCIENCE AND TECHNOLOGY FOR SOCIETAL DEVELOPMENT - IISTSD - 2019

*Jointly organized by Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India*

*&*

*Faculty of Technology, Mahasarakham University, Thailand*

BEST ORAL PRESENTATION AWARD

TO

Mr./Mrs./Dr SOPHIYA . P of Dept. of Studies in Biochemistry, Manglore University, Karnataka for the paper entitled Evaluation of anti-inflammatory . . . . . phase of cancer in the International Conference on 'Impact of Innovations in Science and Technology for Societal Development (IISTSD – 2019)' held during 19 - 21, September 2019 at Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India.

Dr. Narendhirakannan R.T
Convenor
India

Dr. Pariyaporn Itsaranuwat
Convenor
Thailand

Dr. Anuchita Moongngarm
Dean, MSU
Thailand

Dr. M. Leheshmanaswamy
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Dr. C. A. Vasuki
Secretary & Director - KASC
Convenor - ISCA, Coimbatore Chapter